Appendix A

Myocardial ischemia is a condition in which oxygen deprivation to the heart muscle is accompanied by inadequate removal of metabolites because of reduced blood flow or perfusion. During ischemia, an imbalance occurs between myocardial oxygen supply and demand. Ischemia may manifest as (1) anginal discomfort *("angor pectoris")*, (2) EKG changes, (3) reduced uptake in myocardial perfusion images, or (4) regional or global impairment of ventricular function.

By contrast, **myocardial infarction** (MI) is the *irreversible* necrosis of heart muscle secondary to prolonged ischemia. In other words, if the cause of ischemia persists for a period of time (hours) then heart muscle death occurs. MI leads to impairment of systolic function or diastolic function and to increased predisposition to arrhythmias and other long-term complications. **Necrosis**, in turn, is the pathologic death of one or more cells, or of a portion of tissue or organ, resulting from irreversible damage; earliest irreversible changes are mitochondrial, consisting of swelling and granular calcium deposits seen by electron microscopy; most frequent visible alterations are nuclear: pyknosis, shrunken and abnormally dark basophilic staining; karyolysis, swollen and abnormally pale basophilic staining; or karyorrhexis, rupture and fragmentation of the nucleus. After such changes, the outlines of individual cells are indistinct, and affected cells may become merged, sometimes forming a focus of coarsely granular, amorphous, or hyaline material.

Diamond *et al.* (1978) *Am Heart J* 95, 204-9 suggested that ischemic non-infarcted myocardium can exist in a state of functional **hibernation**. Rahimtoola (1989) *Am Heart J* 117, 211-221 proposed the concept of **hibernating myocardium**, as a "chronic *reversible* left ventricular dysfunction due to coronary artery disease".

Following the recognition of the clinical implications of hibernating myocardium, a new concept arose in cardiology, **myocardial viability**, which refers to any myocardial tissue that is *not* infarcted, including myocardial ischemia and hibernating myocardium. In patients with left ventricular dysfunction, revascularization is the treatment of choice only if myocardial viability is

present. That is, improvement of left ventricular dysfunction can only be achieved when myocardial viability is present. Patients with viable myocardium may improve in function after revascularization, whereas patients without viable myocardium do not improve in function. Various non-invasive imaging techniques for assessing viable myocardium and the consequent prediction of improvement in left ventricular function after revascularization have been reported; *e.g.* single photon emission computed tomography (SPECT), as employed in the Vale *et al.* reference. If the presence of ischemia, as well as hibernating myocardium, can be demonstrated, the likelihood of functional recovery after revascularization increases.

Revascularization is a therapeutic method by which physicians enhance myocardial blood flow to the myocardium. It can be achieved by different medical procedures, such as PTCA (percutaneous transluminal coronary angioplasty) and CABG (coronary artery bypass grafting). The augmentation of perfusion, *e.g.* by angiogenesis, is another way to revascularize the myocardium.

Therapeutic revascularization (*e.g.* therapeutic angiogenesis) - in this case, stimulation of the induction of new blood vessels in viable myocardium - would be expected to improve left ventricular function. However, when perfusion (*e.g.*, angiogenesis) is stimulated in non-viable (dead = necrotic = infarcted) myocardium, no improvement of left ventricular function would be expected. Therefore, the revascularization method of Vale *et al.* would not be expected to lead to improvement of left ventricular function. By contrast, therapeutic cardiomyogenesis - the replication of cardiomyocytes - would be expected to improve left ventricular function, not only in viable, but also in non-viable myocardium.

Cardiomyogenesis refers to the replication (mitosis and proliferation) of cardiomyocytes (cardiac muscle cells), which leads to the regeneration of heart tissue. This physiological process can be used as a therapeutic method to regenerate diseased myocardium.

The **NOGA method** employed in Vale *et al.* can distinguish between ischemic and dead (infarcted) tissue; the Vale *et al* paper clearly shows that the tissue they examined was ischemic, but not dead.

The NOGA method is able to assess 2 major parameters to distinguish between normal, ischemic and infarcted tissue: myocardium voltage and function. The patterns are as follows:

- Normal myocardium: normal voltage and function
- Hibernating myocardium: normal voltage and impaired function
- Infarcted myocardium: impaired voltage and function

One of the NOGA results for voltage is called "unipolar endocardial potentials" (UpV). The NOGA result for function is called "linear local shortening" (LLS). For a further discussion of these terms, see, *e.g*, Vale *et al.* at page 966, under the title "LV (NOGA) Mapping" and references 13, 14 and 16 referred to therein.

The three patterns are summarized in the following chart:

	Normal myocardium	Ischemic Myocardium (Hibernating)	Dead Myocardium (Infarcted)
Unipolar endocardial potentials (UpV)	≥5 mV	≥5 mV	< 5 mV
Linear Local Shortening (LLS)	≥12%	4% to 12%	< 4%

In particular, Vale *et al.* states that "Local functional analysis (wall motion) is based on linear local shortening (LLS), a parameter that calculates the fractional shortening of regional endocardial surfaces at end systole. Unipolar (UpV) and bipolar (BpV) endocardial potentials are recorded from the tip electrode, and measurements that are based on these local intracardiac signal amplitudes formulate a guide to myocardial viability. The combination of these 2 data sets permits assessment of electromechanical function that identifies foci of myocardial ischemia. For example, for a given region of interest, UpV ≥5 mV (suggesting viable myocardium) and normal (≥12%) LLS (suggesting normal contraction) would indicate normal myocardium. In contrast, UpV < 5 mV and abnormal (< 4%) LLS (signifying severe regional hypokinesis or akinesis) would indicate a site of LV infarction. Alternatively, UpV ≥5 mV and abnormal LLS of 4% to 12% (indicating mild to

moderate impairment of contractility) would suggest a site of ischemic hibernating myocardium.(13,16)." (p. 966, bottom of column 1, beginning of column 2).

Also, see "Mean UpV and BpV recordings ≥5 mV and ≥2 mV, respectively, defining myocardial viability in the ischemic zone, did not change significantly after **GTx** (Table 2) " (p. 967, col 2, paragraph 2).

The data presented by Vale *et al.* indicate that the tissue being examined was viable myocardium, in particular chronic myocardial ischemia with hibernating myocardium.